

Determination of Plant Bioactive Compounds. Antioxidant Capacity and Antimicrobial Screening

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Abstract

It is well known that bioactive compounds such as polyphenols are constituents of many plants and herbs, and they have attracted a great deal of public and scientific interest because of their health-promoting effects and of their potential applications in foods as antioxidants and antimicrobial agents. Selected plants of interest in traditional diets were examined in order to determine their phenolic composition, antioxidant capacity and antimicrobial activity. Reversed phase high performance liquid chromatography coupled with DAD was employed for the identification and quantification of polyphenols. The phenolic substances were identified and quantified after comparison with reference standards. Catechin, ferulic acid and quercetin were the main polyphenol compounds. The antioxidant capacity of the extracts and of the isolated polyphenol compounds were determined with the Rancimat test using sunflower oil as substrate (the PF factor was determined), the FRAP assay and the DPPH assay (IC₅₀). All plants and isolated compounds exhibited antioxidant capacity. The antimicrobial activity of the extracts against selected microorganisms was also investigated using the disc diffusion method. The plants *Pelargonium purpureum* and *Sideritis scardica* exhibited the most potent antimicrobial activity against all selected pathogenic microorganisms such as *E. coli* and *L. monocytogenes*. These results suggested that the plants selected for this study had properties that required attention for their potential health effect and for possible applications in food products.

Keywords

Phenolic Antioxidants; RP-HPLC; FRAP Assay; DPPH; Rancimat Test; Antimicrobial Activity

Introduction

Lipid oxidation occurring in food products is one of the major concerns in food technology. It is responsible for rancid odors and flavors of the products, with a consequent decrease in nutritional quality and safety, caused by the formation of secondary, potentially toxic compounds. In order to limit these undesirable effects, various methods have been proposed. Besides physical processes, such as oxygen removal and refrigeration, the use of substances that decrease the rate of these oxidation processes plays an important role. Antioxidants, especially those naturally found in plants have been largely viewed as effective tools in the fight against oxidation, whereas in the search for naturally occurring ones, herbs and spices are considered as being one of the most important targets.

This report presents the results on the phenolic composition of Greek plants of the *Lamiaceae* family. The analysis was performed by employing reversed phase high performance liquid chromatography coupled with diode array detection and it was also presented the antioxidant capacity of their methanolic extracts by means of the DPPH and FRAP assay, Rancimat test and their total phenolic content determined spectrometrically by applying the Folin-Ciocalteu assay. The antimicrobial activity of the extracts against selected microorganisms was also investigated using the disc diffusion method.

The outcomes of all determinations were compared and several differences among the phenolic

composition and antioxidant activities of the plant extracts were observed.

Recently, many separation techniques such as gas-liquid chromatography (GLC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) have been proposed to separate and identify phenolic compounds. HPLC and CE, especially coupled with photodiode array detector do not require derivatization prior to qualitative and quantitative analysis. Hence, they have become the most commonly used techniques for the analysis of phenolic compounds in plants. However, often they do not provide satisfactory performance, and the UV-Vis spectrum does not supply sufficient evidence for unambiguous identification. In the present study, the method of Reversed Phase HPLC coupled with a UV-vis Diode Array Detector (DAD) is used. This method enables the collection of on-line spectra and simultaneous quantification in several wavelengths. HPLC-MS is also a useful method for structural identification. Finally the antioxidant capacity as well as the antimicrobial activity of these plant extracts is presented.

Materials and Methods

Standards

Gallic acid, gentisic acid, *p*-coumaric acid, vanillic acid, ferulic acid, syringic acid, (+)-catechin, quercetin, apigenin, naringenin, eriodictyol were purchased from Sigma-Aldrich (Steinheim, Germany). Luteolin was from Roth (Karlsruhe, Germany). Caffeic acid was from Merck (Darmstadt, Germany). (-)-Epicatechin was from Fluka AG (Buchs, Switzerland). Rutin was from Alexis Biochemicals (Lausen, Switzerland). Hydroxytyrosol, *p*-hydroxybenzoic acid and BHT (butylated hydroxytoluene) were a kind donation from the National Agricultural Research Foundation (N.AG.RE.F, Greece). Quantification was done via a calibration with standards (external standard method). All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 62.5% aqueous methanol containing BHT 1 g L^{-1} , and 6M HCL to yield concentrations ranging between $0.5\text{--}25 \text{ mg L}^{-1}$. Stock/ working solutions of the standards were stored in darkness at -18°C .

Solvents and Reagents

Methanol, acetone, acetic acid, dichloromethane were pro analysis (p.a.), acetonitrile and glacial acetic acid

were HPLC grade, and sodium acetate and iron chloride all purchased from Sigma-Aldrich (Steinheim, Germany). Water was supplied by a Milli-Q water purifier system from Milipore (Bedford, MA, USA). The Folin-Ciocalteu reagent was from Merck (Darmstadt, Germany), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Steinheim, Germany), and TPTZ, 2,4,6-Tri(2-pyridyl)-s-triazine was from Fluka (Buchs, Switzerland). The Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany), respectively.

Plant Material

Dried samples were obtained commercially or collected from different sites in Greece. Some samples were dried in the air (at 25°C in the dark) and some were lyophilized. All samples were analyzed within 3 months of collection.

Sample Preparation and Derivatization

The extraction method used for dried samples was as follows: Forty ml of 62.5% aqueous methanol containing BHT (1 g L^{-1}) was added to 0.5g of dried sample. Then 10 ml of 6M HCL were added. The mixture was stirred carefully. In each sample nitrogen was bubbled for ca. 40-60s. The extraction mixture was then sonicated for 15 min and refluxed in a water bath at 90°C for 2h. The mixture was then filtered and made up to 100 ml with methanol. Furthermore, the diluted mixture was filtered quickly through a $0.45 \mu\text{m}$ membrane filter (Millex-HV) and injected to HPLC. To prevent enzymic oxidation, extraction of the polyphenols from plants with boiling alcohol is essential and should be adopted routinely. For this reason all steps were carried out in darkness (flasks were covered with aluminum foil) and under nitrogen atmosphere.

HPLC Analysis

The employed analytical HPLC system consisted of a JASCO high performance liquid chromatograph coupled with a UV-vis multiwavelength detector (MD-910 JASCO). The analytical data were evaluated using a JASCO data processing system (DP-L910/V). The separation was achieved on a Waters Spherisorb® $5\mu\text{m}$ ODS2 $4.6 \times 250\text{mm}$ column at ambient temperature. The mobile phase consisted of water with 1% glacial acetic acid (solvent A), water with 6% glacial acetic acid (solvent B), and water-acetonitrile (65:30 v/v) with 5% glacial acetic acid (solvent C). The

used gradient was similar to that used for the determination of phenolics in wine. The flow rate was 0.5 ml/min and the injection volume was 20 μ l. The monitoring wavelength was 280 nm for the phenolic acids and 320 and 370 nm (flavones, flavonoles). The identification of each compound was based on a combination of retention time and spectral matching.

Rancimat Test

Samples of sunflower oil (3.5g) containing 0.02% w/w extract or 2% w/w ground material were subjected to oxidation at 110 °C (air flow 20 L/h). The standard compounds (0.02% addition) were also examined. Induction periods, *IP* (h), were recorded automatically. The protection factors (PF) were calculated according to the following formula: $PF = IP_{\text{extract}} / IP_{\text{control}}$.

Determination of Total Phenolic Content of Plant Extracts

Total phenolic content was measured by the Folin-Ciocalteu assay. Quantification was performed with the hydrolysed samples. Results were expressed as mg of gallic acid/ g dry sample.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

This widely used decoloration assay was first reported by Brand-Williams and co-workers. This antioxidant assay was based on measurement of the loss of DPPH color at 517 nm after reaction with test compounds and the reaction was monitored by a spectrometer. Experiments were carried out according to with a slight modification. Briefly, a 1mmol/l solution of DPPH radical solution in methanol was prepared and then, 1 ml of this solution was mixed with 3 ml of sample solution in different concentrations after extraction with different solvents (methanol, water, dichloromethane). Dichloromethane was removed in a rotary evaporator at 40 °C and the residue was dissolved in methanol. After 30 min, the absorbance was measured at 517 nm. The % DPPH radical scavenging was calculated from the equation:

$$\% \text{DPPH radical-scavenging} = [(Ab_{\text{Scontrol}} - Ab_{\text{Ssample}}) / Ab_{\text{Scontrol}}] \times 100.$$

The DPPH solution without sample solution was used as control. The Inhibitory Concentration 50 (*IC*₅₀) value of extracts (the concentration that causes a decrease in the initial DPPH concentration by 50%) was calculated by using the calibration %DPPH radical-scavenging = f(concentration (μ g/ml) and expressed in μ g/ml. Results were compared with ascorbic acid and BHT,

which were used as standards.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was originally developed by to measure reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals. The assay involves the following procedures: The oxidant is prepared by mixing TPTZ (2.5 ml, 10 mmol/l in 40 mmol/l HCl), 25 ml of acetate buffer, and 2.5 ml of FeCl₃·H₂O (20 mmol/l). The conglomerate is referred to as "FRAP reagent". To measure FRAP value, 300 μ l of freshly prepared FRAP reagent is warmed to 37 °C and a reagent blank reading is taken at 593 nm; then 10 μ l of sample (extracted with water, ethanol, 60% ethanol and methanol) and 30 μ l of water are added. Absorbance readings are taken at 0, 4, and 30 min. The change of absorbance ($\Delta A = A_{4\text{min}} - A_{0\text{min}}$ and $\Delta A = A_{30\text{min}} - A_{0\text{min}}$) is calculated.

Statistical Analysis

Statistical analysis was performed using Minitab 13.1 for Windows. The mean values obtained in the different studied samples were compared by one way analysis of variance (MANOVA). Tukey's test was then used to determine which mean values were different.

Antimicrobial Assay

The methanolic extracts were tested against a panel of pathogenic microorganisms, including *Escherichia coli* 0157:H7 NCTC 12900, *Salmonella enteridis* PT4, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ScottA, *Pseudomonas putida* AMF178 and *Bacillus cereus* FSS134. Microorganisms were stored frozen in bead vials (Protect; Technical Service Consultants Ltd. Heywood, Lancashire, UK). Resuscitation of bacterial strains was carried out in 10 ml BH Broth (Merck cat.no.1.10493) incubated overnight at 37°C for *Escherichia coli* and *Salmonella enteridis* PT4, 35°C for *Bacillus cereus*, 30°C for *Listeria monocytogenes* and *Staphylococcus aureus* and 25°C for *Pseudomonas putida*.

Resuscitated cultures were diluted tenfold in Ringer's solution (LabM) for the inoculation of 10 ml BH Broth (Merck cat.no.1.10493) to yield an initial suspension of approximately 10 to 100 cfu/ml. All broths were then incubated statically at the aforementioned temperatures for each microorganism, for 18-24 h to guarantee that all cells were in the stationary phase.

Susceptibility of the test organism to the extract was determined by employing the standard disk diffusion technique. The bacterial suspensions were diluted tenfold in Ringer's solution (LabM), and 0.1 ml from the appropriate dilution was spread plated on BH Agar (Merck cat.no.1.13825) in order to give a population of approximately 10^6 cfu/plate. Sterile paper discs with a diameter of 6.48mm (Whatman No 2) were placed onto the inoculated agar surface. Five μ l of each plant extract was added to the paper discs. Each experiment was carried out in triplicate. Petri dishes were incubated for 48 h., at 37°C for *E. coli* 0157:H7 NCTC 12900 and *Salm. Enteritidis* PT4, 35°C for *B. cereus* and 30°C for *L. monocytogenes* ScottA and *S. aureus* ATCC 6538 and 25°C for *Pseudomonas putida*. After incubation, the inhibition zones were estimated by taking photos of Petrie's with a SONY camera (x-wave HAD SSC-DC50AP) and processed using the Impuls Vision XL 2.5 software. Each inhibition zone diameter was measured three times (three different plates) and the average was taken.

Results and Discussion

Antioxidant Capacity

The antioxidant capacity (expressed as PF values) and the total phenolic content of all extracts are shown in TABLE 1. The highest amount was found in *Salvia officinalis*, and the lowest in *Sideritis scardica*. Similar amount in plant phenolics from herbs and medicinal plants collected in Finland have been reported recently.

The outcome of the Rancimat test supports the hypothesis that aromatic plants are good sources of natural antioxidants such as phenolic compounds. When ground material was added to sunflower oil, protection factors were slightly higher compared to the addition of methanol extracts. The examined PF values for the standard compounds (0.02% addition) ranged from 1.2 to 1.5 for all phenolic acids, except for gallic acid which had PF = 4.5. (+)-Catechin hydrated and (-)-epicatechin had PF values 1.8 and 2.5 respectively, whereas the flavonoids (rutin, quercetin, apigenin, luteolin, eriodictyol and naringenin) had PF values ranging from 1 to 1.2. The PF value for hydroxytyrosol was 1.4 and 1.8 for butylated hydroxytoluene. These values explained the antioxidant potential of the plant extracts examined and depend on the total phenol content, without being proportional. Similar PF values for ethanol and acetone extracts of plants of Greek origin have been

reported. A wide range of color reactions has been used to determine total polyphenols, including the reaction with the Folin-Ciocalteu reagent. However, as each phenolic compound giving a different color and assays in general will be rather unspecific, the results of these determinations are of limited value.

HPLC Analysis

Using the aforementioned procedure, the phenolic substances in aromatic plants of Greek origin were separated and quantified. HPLC with UV-vis multiwavelength detector was used since all phenolic compounds showed intense absorption in the UV region of the spectrum. The present method is simple, easy to use, and effective enough for the identification and quantification of major phenolic compounds in aromatic plants. A similar technique has been reported by other authors, for the analysis of major flavonoid aglycones. After extraction and acid hydrolysis the content of phenolic substances was determined. Quantification was done via a calibration with standards (external standard method). The amount of phenolic acids detected in the analysed samples is shown in TABLE 2. Additionally, the content of flavonoids identified in the same plant extracts is shown in Table 3. Results are expressed in mg/ 100g dry sample. Another phenolic compound which was detected as well in some samples was hydroxytyrosol. Hydroxytyrosol, is one of the main bioactive constituent in olives. *Geranium purpureum* contained a large amount of this compound (11.2 mg /100g dry sample). In the majority of other kinds of plant extracts hydroxytyrosol was detected in traces. The most abundant phenolic acids were ferulic (4.1 – 13.2 mg /100g dry sample) and caffeic acid (4.9 – 20 mg /100g dry sample). *Filipendula ulmaria* contained the highest amount of ferulic acid (13.2 mg /100g dry sample). Syringic acid was detected only in *Geranium purpureum*. (+)-Catechin, rutin (quercetin 3-o-rhamnose glycoside) and quercetin were the most abundant flavonoids. Apigenin was detected only in *Salvia officinalis*. (TABLE 3). The levels of phenolic compounds were expected according to previous investigations of these compounds in similar herbs, plants. The flavonol quercetin and the two major flavones luteolin and apigenin have been identified and simultaneously quantified in 62 types of edible tropical plants in Malaysia.

The data presented in TABLES 1, 2 and 3 are considered as indicative of phenolic content of these

aromatic plants. Papers of most of the examined plant extracts are very limited in the literature. Among others, time of harvest, storing conditions, are considered responsible for the observed variations in the phenolic contents.

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Assay

The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. IC_{50} values of hop extracts were found to be similar to BHT and ascorbic acid values (0.51 ± 0.03 and 16.3 ± 0.02 mg/L) as shown in TABLE 1. In the same table the IC_{50} values of some standard phenolic compounds are also presented. All the IC_{50} values of the plant extracts (extracted with methanol, water, and dichloromethane) were determined with the use of curves plotted as the ones of *Salvia officinalis* shown in FIGURE 1. These curves show the % DPPH radical scavenging against concentration ($\mu\text{g/ml}$). Methanol extract has given better antioxidant capacity compared to other solvent extracts. DPPH is a stable nitrogen radical that bears no similarity to the highly reactive and transient peroxy radicals involved in lipid peroxidation.

FRAP (Ferric Reducing Antioxidant Power)

The FRAP mechanism is totally electron transfer rather than mixed SET and HAT, so in combination with other methods can be very useful in distinguishing dominant mechanisms with different antioxidants. In addition, because reduced metals are active propagators of radical chains via hydroperoxide reduction to $RO\bullet$, it would be interesting to evaluate whether high FRAP values correlate with the tendency of polyphenols to become pro-oxidants under some conditions. This has been shown for some flavones and flavanones which also have high FRAP values. The experiments were based on the measurement of the absorbance of the plant extracts after extraction with four different solvents at time 0 (beginning of the measurement) and after 4 and 30 min. The change of absorbance ($\Delta A = A_{4\text{min}} - A_{0\text{min}}$ and $\Delta A = A_{30\text{min}} - A_{0\text{min}}$) was calculated. In FIGURE 2 the change in absorbance from 0 to 30 minutes for all solvent extracts is presented, as well as with the effect of the solvent to the change of absorbance (ΔA) for the two time measurements (4 and 30 min). It can be seen from the results that the change in absorbance between 0 and 30 min for the 60% ethanol extract is significantly higher ($p < 0.05$) than the other extracts. Between 0 and 4 min the ethanol extract has significantly lower ($p < 0.05$) change in absorbance than the other extracts. There is

also a significant difference ($p < 0.05$) in ΔA for the methanol extracts after 4 and 30 min. No other significant difference was observed. 60% ethanol and methanol extracts changed significantly the reducing capacity of antioxidants based upon the ferric ion. The order of reactivity of a series of antioxidants can vary tremendously and even invert, depending on the analysis time. These authors recently examined the FRAP assay of dietary polyphenols in water and methanol. The absorption (A_{593}) slowly increased for polyphenols such as caffeic acid, tannic acid, ferulic acid, ascorbic acid, and quercetin, even after several hours of reaction time. Thus, a single-point absorption endpoint may not represent a completed reaction. In contrast to other tests of total antioxidant capacity, the FRAP assay is simple, speedy, inexpensive, and robust and does not require specialized equipment.

Antimicrobial Activity

To screen the antimicrobial activity of "unknown compounds" the disk diffusion method is considered to be the simplest method where the results are obtained rapidly. Disk diffusion method has been used by to determine the antimicrobial activity of essential oil and methanol extracts using several microbial strains. Antimicrobial activity of *Decalepis hamiltonii* roots against foodborne pathogens using the same method has been reported.

The antimicrobial activity of plant extracts are shown in TABLE 4. The plant extracts found to be more effective on inhibiting the microorganisms tested were: *Geranium purpureum*, *Sideritis scardica* and *Rosmarinus officinalis*. *Listeria monocytogenes* "ScottA" was the most sensitive microorganism to the plant extracts examined in this study. On the contrary, all the extracts failed to inhibit *Salmonella enteridis* PT4. The results in TABLE 4 revealed that Gram (+) bacteria was more sensitive to the plant extracts than Gram (-) bacteria especially the Enterobacteriaceae (*Escherichia coli* 0157:H7 NCTC12900 and *Salmonella enteridis* PT4). It should be taken into account that the comparatively weak inhibition found in this study could be influenced by the fact that the inhibition area depending on the ability of the antimicrobial compound to diffuse uniformly through the agar. Thus, a greater inhibition might be revealed if alternative(s) methodologies were applied.

Conclusions

The current study showed that all plants and isolated compounds exhibited antioxidant capacity. The

antioxidant capacity was determined with the Rancimat test using sunflower oil as substrate, the FRAP assay and the DPPH assay (by using the IC₅₀ value). The antimicrobial activity of the extracts against selected microorganisms was also investigated using the disc diffusion method. Gram (+) bacteria was more sensitive to the plant extracts than Gram (-) bacteria especially the Enterobacteriaceae (*Escherichia coli* 0157:H7 NCTC12900 and *Salmonella enteridis* PT4). The plants *Pelargonium purpureum* and *Sideritis scardica* exhibiting the most potent antimicrobial activity against all selected pathogenic microorganisms such as *E. coli* and *L. monocytogenes*. Catechin, ferulic acid and quercetin were determined by HPLC analysis as the main polyphenol compounds in the plant extracts. The obtained results indicated that the analyzed plant extracts may become important, cheap and noticeable sources of bioactive compounds, such as phenolic compounds, with antioxidant and antimicrobial activity simultaneously and have potential use in the food industry.

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TABLE 1 TOTAL PHENOLICS IN PLANT EXTRACTS AND THEIR ANTIOXIDANT CAPACITY (EXPRESSED AS PF VALUES AND IC₅₀)

Species	Part examined	Drying method ^a	Total phenolics ^b (mg gallic acid/ g ds)	PF ^c (ground material)	PF (methanol extracts)	IC ₅₀ ^d (mg/L)
<i>Filipendula ulmaria</i>	Flower	Air	77.4 ± 3.71	1.3	1.2	47.0 ± 2.1
<i>Salvia officinalis</i>	Herb	Air	234.7 ± 12.9	1.5	1.4	23.4 ± 1.4
<i>Rosmarinus officinalis</i>	Herb	F / v	190.2 ± 6.3	1	0.8	34.9 ± 2.2
<i>Sideritis scardica</i>	Leaves	F / v	75.3 ± 2.1	1.8	1.7	49.6 ± 0.4
<i>Geranium purpureum</i>	Leaves	F / v	120.5 ± 3.7	3.1	2.9	41.3 ± 2.1

^a Air = air drying; F / v = Freeze vacuum, i.e. lyophilization. ^b Mean of duplicate assays; ds = dry sample; ^c PF = protection factor; ^d IC₅₀ = Inhibitory Concentration 50

TABLE 2 CONTENT OF PHENOLIC ACIDS IN THE EXAMINED PLANT EXTRACTS

Plant	Content, mg/ 100g dry sample ^a							
	gallic acid	gentisic acid	caffeic acid	<i>p</i> -coumaric acid	vanillic acid	Syringic acid	ferulic acid	<i>p</i> -hydroxybenzoic acid
<i>Filipendula ulmaria</i>	ND	ND	6.5 ± 0.02	4.3 ± 0.02	4.8 ± 0.02	ND	13.2 ± 0.04	ND
<i>Salvia officinalis</i>	3.4 ± 0.01	ND	ND	ND	ND	ND	6.7 ± 0.02	ND
<i>Rosmarinus officinalis</i>	1.2 ± 0.02	ND	4.9 ± 0.01	ND	ND	ND	5.8 ± 0.02	ND
<i>Sideritis scardica</i>	3.7 ± 0.01	ND	5.6 ± 0.02	ND	ND	ND	4.1 ± 0.01	ND
<i>Geranium purpureum</i>	14 ± 0.02	3.2 ± 0.03	20 ± 0.03	4.1 ± 0.02	2 ± 0.02	1.1 ± 0.02	ND	5.4 ± 0.01

^a Each value is the mean (mg / 100g dry sample) of two replications ± standard deviation; ND = not detected

TABLE 3 FLAVONOID CONTENT IN THE EXAMINED PLANT EXTRACTS

Plant	content, mg/ 100g dry sample ^a							
	quercetin	apigenin	luteolin	naringenin	eriodictyol	rutin	(+)-catechin hydrated	(-)-epicatechin
<i>Filipendula ulmaria</i>	1.5 ± 0.01	ND	ND	ND	ND	1.3 ± 0.01	ND	ND
<i>Salvia officinalis</i>	3.6 ± 0.01	4 ± 0.01	ND	1.7 ± 0.02	ND	2.6 ± 0.01	ND	ND
<i>Rosmarinus officinalis</i>	ND	ND	3.1 ± 0.02	ND	0.3 ± 0.02	ND	ND	ND
<i>Sideritis scardica</i>	3.6 ± 0.02	ND	ND	ND	ND	1.9 ± 0.01	ND	4.5 ± 0.04
<i>Geranium purpureum</i>	11.2 ± 0.02	ND	ND	ND	ND	4.5 ± 0.01	1.5 ± 0.01	5.6 ± 0.02

^a Each value is the mean (mg / 100g dry sample) of two replications ± standard deviation; ND = not detected

TABLE 4 ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS (SAMPLE AMOUNT 5µl; n = 3)

Plant extracts	<i>Escherichia coli</i> 0157:H7 NCTC12900	<i>Salmonella enteridis</i> PT4	<i>Staphylococcus aureus</i> ATCC 6538	<i>Listeria monocytogenes</i> ScottA	<i>Bacillus cereus</i> FSS134	<i>Pseudomonas putida</i> AMF178
<i>Filipendula ulmaria</i>	- ^a	-	~ ^b	++ ^d	~	+ ^c
<i>Salvia officinalis</i>	~	-	-	++	~	~
<i>Rosmarinus officinalis</i>	-	-	-	+	-	+
<i>Sideritis scardica</i>	+	-	-	++	-	+
<i>Geranium purpureum</i>	++	-	+	++	+	++

^a - : No antimicrobial capacity, i.z. of sample < i.z. of solvent (62.5% aqueous methanol)

^b ~: Slight antimicrobial capacity, i.z. of sample 1 – 3 mm > i.z. of solvent

^c +: Moderate antimicrobial capacity, i.z. of sample 3 – 4 mm > i.z. of solvent

^d ++: Clear antimicrobial capacity, i.z. of sample 4 – 10 mm > i.z. of solvent

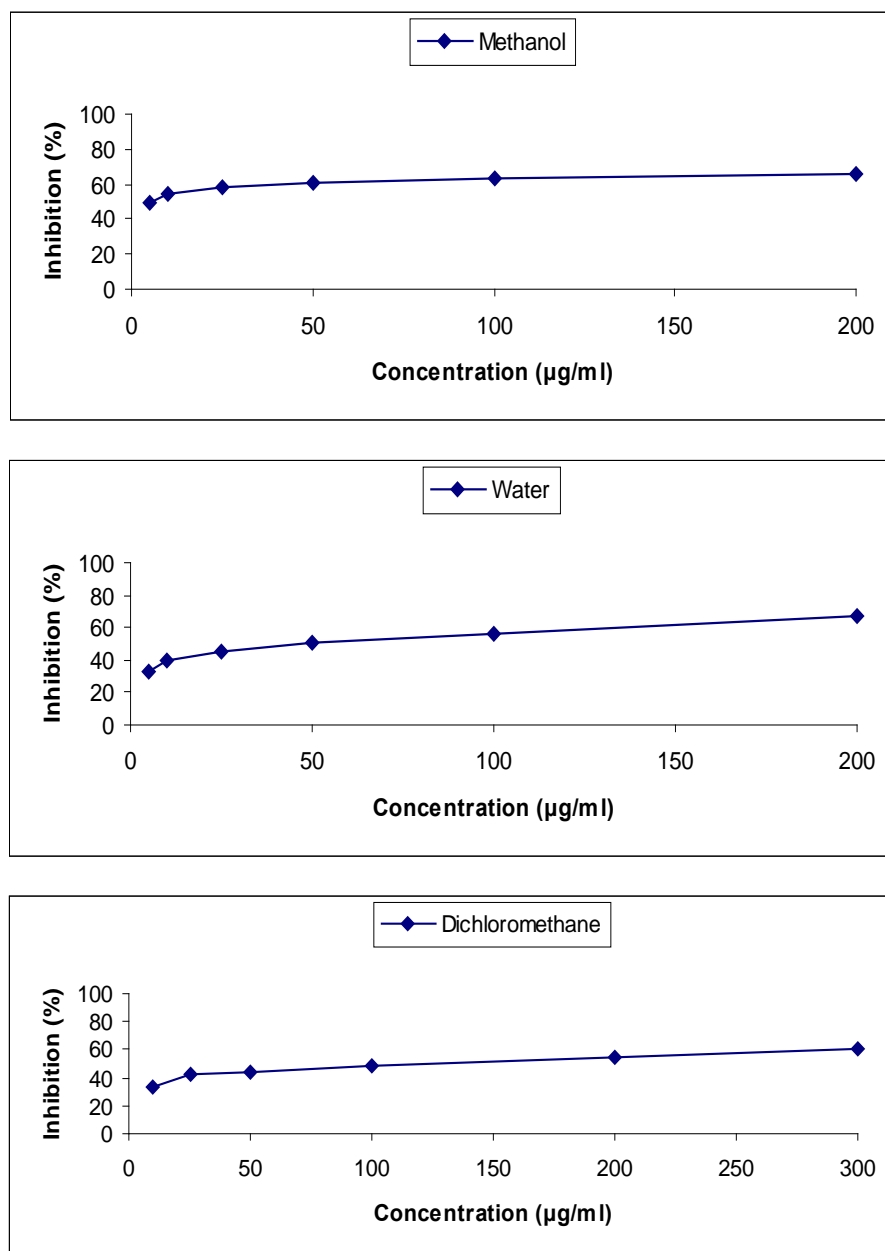
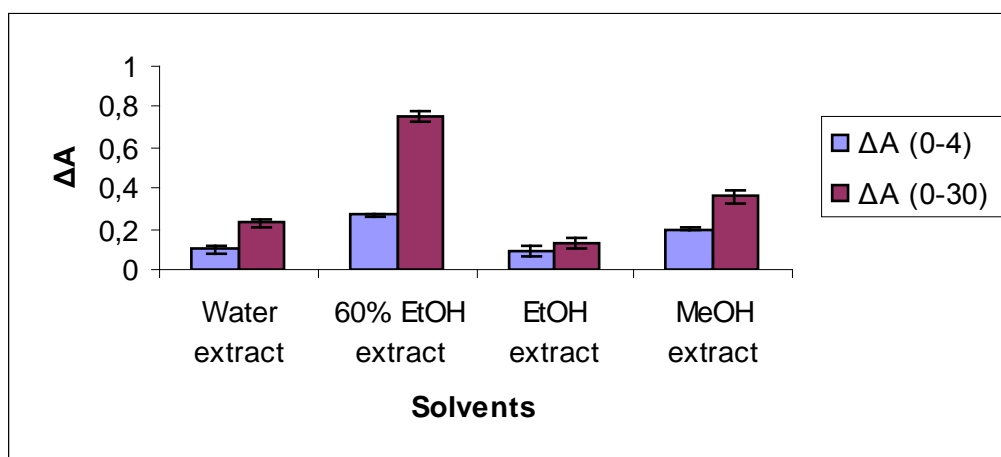


FIGURE 1 % DPPH RADICAL SCAVENGING AGAINST CONCENTRATION (MG/ML) CURVES USED TO DETERMINE THE IC₅₀ VALUES OF *SALVIA OFFICINALIS* EXTRACT



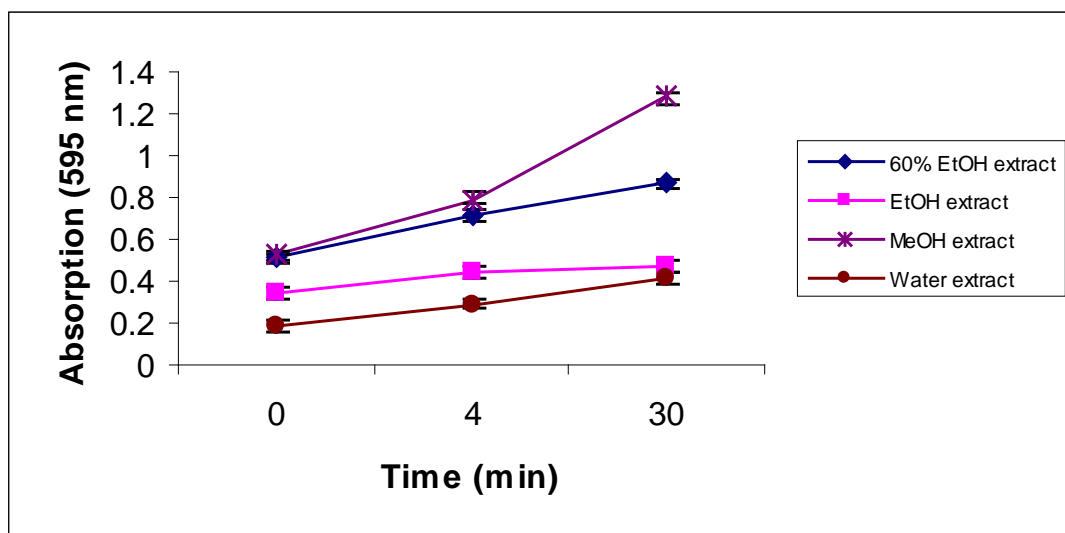


FIGURE 2 CHANGE OF ABSORBANCE ($\Delta A = A_{4\text{MIN}} - A_{0\text{MIN}}$ AND $\Delta A = A_{30\text{MIN}} - A_{0\text{MIN}}$) AND ABSORPTION READINGS TAKEN AT 0, 4, AND 30 MIN FOR *SALVIA OFFICINALIS* EXTRACT